The Effect of Nitrogen-fixing Organisms and Nucleic Acid Derivatives on Plant Growth.

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Introduction.

In a previous communication* it has been shown that a water extract of bacterised peat has a remarkable effect on the growth of *Lemna minor* in water-culture solution. The experiments there described showed that when supplied with inorganic nutrients only, the plants, though increasing in number, failed to maintain their normal size and health, and rapidly deteriorated, while the addition of a small quantity of the organic material resulted in a marked increase in the rate of multiplication, the plants at the same time showing improved health and vigour, proof of which was given by the increase in the dry weight of the plants so treated.

The author has also shown† that wheat seedlings, when deprived of their endosperm at a very early stage, will not grow normally unless supplied with organic matter. These experiments led to the conclusion that all plants, as well as animals, require a certain amount of *organic* substance for their proper development—a conclusion entirely contrary to the established view that plants can be grown in purely inorganic nutrients in water-culture.

In the course of the previous work, however, it was found that the organic matter is effective in extremely minute quantities, such as might conceivably be supplied by bacterial and algal contamination, though in these preliminary stages no attempt was made to determine the nature of the essential substances. The water extract of the bacterised peat, which was largely used, must be a complex mixture, containing constituents from both the peat and the bacteria, as well as the products of their interaction; and the growth-promoting effect may be due to any or all of these substances. The all-important bacteria used in the preparation of the bacterised peat are those concerned in nitrogen-fixation, and in May, 1917, a preliminary experiment was carried out with the object of ascertaining how far the effect of the bacterised peat extract was due to the products of these bacteria.

* Bottomley, W. B., 'Roy. Soc. Proc.,' B, vol. 89, pp. 481–507 (1917).

† Bottomley, W. B., 'Annals of Botany,' vol. 28, No. 111, pp. 531–540 (1914).

Effect of Azotobacter chroococcum.

Two series of five dishes each were arranged; the dishes in Series I, numbered from 1 to 5, each containing 250 c.c. of Detmer's solution, and those in Series II, numbered from 6 to 10, each containing 250 c.c. of the same solution, with the addition of 0·1 grm. of a growth of *Azotobacter chroococcum* in every 100 c.c. This growth was obtained by making streak cultures on agar-mannite plates from a pure colony of the organism, and incubating for about 14 days. At the end of this period a copious brown gelatinous growth was obtained over practically the whole surface, which was carefully scraped off with a spatula, transferred to a tared beaker, and rapidly weighed. The mass in the beaker was covered with distilled water, and the whole was raised to a temperature of 120° C. for one hour in an autoclave. The contents of the beaker were then measured and transferred to a well-stoppered bottle, the beaker and measuring cylinder being repeatedly rinsed out with measured quantities of distilled water, and the washings added to the bottle, until a volume of about 10 c.c. for every gramme of the gelatinous growth had been obtained. The stoppered bottle was placed in a shaking machine, and well shaken for six hours, at the end of which time the gelatinous mass appeared to be completely broken up and a uniform suspension obtained. A few drops of chloroform were added to this to ensure sterility.

When required for use, an aliquot portion of this liquid, containing the requisite quantity of bacterial growth, was measured out after the bottle had been well shaken, transferred to a beaker together with some distilled water and heated on a water-bath to a temperature of about 80° C. to remove the chloroform. When cold, the concentrated Detmer's solution and distilled water in sufficient quantity to make up the required volume were added. The Detmer's solution was prepared in quantity at one hundred times the normal concentration and kept in a stock bottle. These experiments were carried out entirely with conductivity water.

Ten similar plants of *Lemna minor* were counted out into each of the ten dishes, and 300 plants counted out at the same time for a determination of their dry weight. The dishes were covered with black paper to the surface of the liquid, as described in the previous communication, and the whole set placed in a greenhouse. The dishes were protected from dust by a large sheet of glass, supported at a height of about 2 in. above the top of the dishes. The culture solutions were changed twice weekly, and the plants counted once weekly. At the end of the third week the plants in Series II had almost filled their dishes, so the whole set was halved at the weekly

counting, and this was repeated each week until the end of the experiment. When necessary the sets were even quartered, three-fourths of each dish being discarded. The figures obtained are shown in the Table below, the numbers given being the total numbers from the original ten plants in each dish, and not the fractions retained at the weekly countings.

Table I.

Series.	No.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.
I.							
Detmer's Solution ...	1	22	80	212	576	1,248	1,984
	2	31	79	174	416	1,056	1,568
	3	30	82	166	464	1,024	1,696
	4	32	82	194	432	1,088	1,824
	5	35	86	210	512	1,248	1,824
Mean	—	32·0	81·8	191·2	480	1,132·8	1,779·2
II.							
Detmer's Solution + *Azotobacter*	6	40	135	532	2,624	10,240	25,120
	7	42	142	502	2,656	12,224	27,776
	8	37	130	564	2,720	10,656	27,872
	9	36	127	478	2,512	11,808	24,064
	10	43	159	600	2,848	11,040	28,832
Mean	—	39·6	138·6	535·2	2,672	11,193·6	26,732·8

At the end of the experiment the plants from three dishes in each set were used for a determination of the dry weight, and from the figures so obtained the dry weight of 100 plants was calculated. The results are shown below :—

	Mgrm.
Dry weight of 100 plants at beginning of experiment	14·8
„ „ from Series I at end of experiment ...	10·0
„ „ from Series II „ „ ...	18·2

It is evident from these figures that the addition of the sterilised *Azotobacter chroococcum* to the culture solution not only resulted in an increased rate of multiplication of the Lemna plants, but also enabled them to increase their original size, while the weight of the control plants showed a corresponding decrease. It therefore appears that part, at least, of the beneficial effect of the water extract of bacterised peat is due to the products of the nitrogen-fixing bacteria which it contains ; and if such an effect as that recorded above be produced by a definite addition of bacterial substance, it is

not unreasonable to suppose that a similar result might be attained in ordinary water-culture experiments by natural bacterial contamination, which is almost unavoidable unless the most scrupulous care be taken to ensure the sterility of the solutions and to change them frequently. It should be pointed out that the gelatinous growth of *Azotobacter* contained 96·68 per cent. of moisture, so that the addition of this material in the proportion of 1 grm. to 1,000 c.c. of solution represents only 33·2 parts of dry substance per million, containing 29 parts of organic matter. This quantity could quite conceivably be supplied by bacterial and algal contamination, and it is interesting in this connection to point out that it has been frequently observed that a more luxuriant growth is obtained in water-culture experiments when the solutions become contaminated with green algæ.

Effect of Nucleic Acid Derivatives.

It was not to be expected, however, that the whole of the effect of the bacterised peat extract was to be attributed to the products of these nitrogen-fixing bacteria; and since the effect of this organic extract was most marked upon the nuclei of the young developing plants, it was suggested that some nuclear constituent, present in the peat and rendered available during the process of "bacterisation," might be partially responsible for the results obtained.

It has already been shown* that on extracting raw peat with dilute alkalies and removing the "humic acids" by suitable means, certain nucleic acid derivatives can be obtained; and that by extracting the peat repeatedly with a 1 per cent. solution of sodium bicarbonate, these same nucleic acid derivatives are obtained, but the "humic acids" are not dissolved, so that the trouble of removing these latter substances is avoided. The nucleic acid derivatives thus obtained consist generally of an adenine-uracil dinucleotide and two mononucleotides—a guanine and a cytosine mononucleotide. The dinucleotide can be precipitated from the sodium bicarbonate extract, after concentration *in vacuo*, by an excess of absolute alcohol following the addition of sodium acetate and hydrochloric acid to the extract. A flocculent precipitate is obtained, which settles after about 24 hours to a fine powder, and this can be filtered off and washed with absolute alcohol. The two mononucleotides remain in the filtrate.

In order to test the effect of these various substances on the growth of *Lemna minor*, a weighed quantity of peat was repeatedly extracted with successive portions of a 1 per cent. solution of sodium bicarbonate until the extract was no longer coloured. The combined extracts, after carefully

* Bottomley, W. B., 'Roy. Soc. Proc.,' B, vol. 90, pp. 39–44 (1917).

neutralising with hydrochloric acid, were concentrated *in vacuo* and divided into two equal portions, to one of which a few drops of chloroform were added, in order to prevent bacterial growth. This liquid was preserved in a bottle and known as " crude nucleic acid derivatives from raw peat." To the other half a little sodium acetate was added, and sufficient hydrochloric acid to render the liquid acid to litmus, and then about twice its volume of absolute alcohol to completely precipitate the dinucleotide. After 24 hours the liquid was decanted off through a filter, the precipitate washed with a little absolute alcohol, allowed to settle, and the liquid again decanted. This was repeated until the alcohol was no longer coloured. The precipitate was then dried *in vacuo* and dissolved up in a very slight excess of sodium carbonate solution, the excess being finally carefully neutralised with dilute hydrochloric acid. When made up to a known volume a few drops of chloroform were added, and the liquid, bottled for use, was known as " adenine-uracil dinucleotide from raw peat."

An experiment was then made in June, 1917, to test the effect on the growth of *Lemna minor* of each of these fractions from raw peat, in comparison with that of *Azotobacter chroococcum* and of bacterised peat. A set of thirty dishes was arranged in six series of five dishes each, and all contained 150 c.c. of Detmer's solution with the following additions:— Series I, numbered from 1 to 5, no addition; Series II, numbered from 6 to 10, the crude nucleic acid derivatives from 1 grm. of raw peat in every 500 c.c.; Series III, numbered from 11 to 15, the adenine-uracil dinucleotide from 1 grm. of raw peat per 500 c.c.; Series IV, numbered from 16 to 20, 0·5 grm. of the autoclaved growth of *Azotobacter chroococcum* per 500 c.c.; Series V, numbered from 21 to 25, the crude nucleic acid derivatives from 1 grm. of raw peat plus 0·5 gram of the growth of *Azotobacter chroococcum* per 500 c.c., *i.e.*, the addition made in Series II plus that made in Series IV; and Series VI, numbered from 25 to 30, the water extract of 1 grm. of bacterised peat per 500 c.c.

When the various extracts were required for use, the requisite quantities of the respective liquids were measured out from the stock bottles, transferred to evaporating basins, and warmed on the water-bath to a temperature of 80° C. to expel the chloroform. When cold they were added to the concentrated Detmer's solution and made up to the required volume with conductivity water.

Ten plants of *Lemna minor* were counted out into each dish, 300 similar plants being counted out at the same time for an estimation of their dry weight. The dishes were covered with paper to the level of the liquid, as described above, to cut out the light from the bottom and sides, and placed in

a greenhouse as before. The solutions were changed twice weekly and the plants counted once weekly, and when they had filled their dishes in some of the series, as they had at the end of the second week in Series V and VI, the number throughout the set was halved at the weekly counting, only one-half of each dish being retained. This was repeated each week throughout the experiment, which lasted six weeks, the plants being even quartered when necessary. Table II shows the figures obtained, which represent the total numbers of plants arising from the original ten in each dish :—

Table II.

Series.	No.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.
I.							
Detmer's Solution ...	1	29	84	256	352	704	1,472
	2	33	92	224	384	832	2,176
	3	32	96	208	384	704	1,536
	4	34	96	176	416	1,024	2,112
	5	33	88	208	384	768	1,792
Mean	—	30·2	91·2	214·4	384·0	806·4	1,817·6
II.							
Detmer's Solution + Crude Nucleic Acid Derivatives	6	44	212	900	3,008	16,896	109,824
	7	46	242	904	3,304	18,248	104,416
	8	39	238	836	3,160	16,568	84,528
	9	38	220	832	3,264	16,128	92,400
	10	47	244	864	2,880	14,848	93,440
Mean	—	42·8	231·2	867·2	3,123·2	16,537·6	96,921·6
III.							
Detmer's Solution + Dinucleotide	11	40	184	560	1,792	7,936	33,024
	12	41	196	640	1,984	7,168	30,208
	13	41	160	496	1,536	6,912	27,904
	14	36	172	560	1,728	8,448	35,584
	15	38	184	608	1,664	6,912	31,232
Mean	—	39·2	179·2	572·8	1,740·8	7,475·2	31,590·4
IV.							
Detmer's Solution + Autoclaved *Azotobacter*	16	40	168	592	2,048	12,288	69,376
	17	40	232	816	2,752	16,128	81,920
	18	45	208	784	3,072	18,432	91,136
	19	41	240	736	2,496	14,592	73,728
	20	42	212	784	2,944	17,664	84,736
Mean	—	41·6	212·0	742·4	2,662·4	15,820·8	80,179·2

Table II—*continued.*

Series.	No.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.
V.							
Detmer's Solution + Crude Nucleic Acid Derivatives + *Azotobacter*	21	52	356	1,504	6,912	44,032	299,008
	22	50	344	1,680	7,936	40,448	277,504
	23	55	344	1,520	6,912	40,960	274,432
	24	45	320	1,472	7,232	44,544	323,072
	25	52	360	1,712	6,592	37,888	291,840
Mean	—	50·8	344·8	1,577·6	7,116·8	41,574·4	293,171·2
VI.							
Detmer's Solution + Bacterised Peat	26	50	324	1,536	9,088	47,104	311,808
	27	50	344	1,552	8,256	37,376	253,952
	28	55	372	1,856	8,128	41,472	302,592
	29	46	364	1,728	8,448	41,472	294,912
	30	50	336	1,296	7,552	38,400	301,056
Mean	—	50·2	348·0	1,593·6	8,294·4	41,164·8	292,864·0

From the second week onwards, when the plants were halved or quartered at the weekly countings, the discarded fractions from the five dishes in each series were added together and thoroughly washed, in order that an estimation of the dry weights of the plants could be made. From the data thus obtained a calculation was made of the dry weight of 100 plants in each series week by week, and a comparison of these weekly weights with the original weight of the plants is shown in the Table below :—

Table III.

Series No.	Weight of 100 plants in milligrams.					
	At beginning.	2nd week.	3rd week.	4th week.	5th week.	6th week.
I	16·4	16·7	11·0	9·6	4·9	4·7
II	16·4	19·3	18·7	20·1	18·1	18·6
III	16·4	19·3	18·4	17·9	18·8	17·7
IV	16·4	19·1	18·1	18·9	19·9	18·7
V	16·4	18·6	18·3	20·7	17·9	19·8
VI	16·4	19·6	18·6	17·1	20·0	19·7

The slight fluctuation shown in the weights of the plants in some series week by week, instead of a steady rise or fall, is explained by the irregular size of the small plants formed during the rapid multiplication of the large

numbers obtained, resulting in a variable average weight for the series concerned.

It should be pointed out here that the increase in number of the plants in this experiment does not correspond with the figures given in Table I for a similar period. However, all experiments reported were carried out at different times, and each one is as complete as possible within itself, for it is impossible to make any correct comparisons between trials carried out at various periods of the year, owing to the great variation of such factors as duration of sunlight, temperature, etc.—factors which have a very great influence on the rate of growth and multiplication of the plants.

An examination of the numbers given in Table II shows that both the crude nucleic acid derivatives in Series II and the autoclaved *Azotobacter* in Series IV have the effect of markedly increasing the rate of growth and multiplication of the Lemna plants; and the figures in Table III show that the average weights of the individual plants in the same series increased beyond their original weight, while the weight of the plants in the control series steadily decreased. It is evident, therefore, that both the crude nucleic acid derivatives and the *Azotobacter* have a growth-promoting effect; but when the two are added together to the Detmer's solution, as in Series V, their combined effect is far greater than the sum of their effects when added separately, as in Series II and IV, and is approximately equal to that of the water extract of bacterised peat in Series VI. It would therefore appear that the growth-promoting substances in these two liquids are dissimilar in their action upon the plant, and that they are in some manner complementary to one another; for were they similar in their *rôle* in the plant metabolism, it would be expected that the effect of the two when supplied together would be approximately equal to the sum of their separate effects.

The remarkable similarity between the results produced by the extract from 1 grm. of bacterised peat, on the one hand, and the crude nucleic derivatives from 1 grm. of raw peat, together with the *Azotobacter* growth, on the other, is readily explained. It may reasonably be supposed that the water extract from 1 grm. of bacterised peat contains a quantity of the nucleic acid derivatives approximately equal to that in 1 grm. of raw peat, these substances having been rendered water-soluble during the process of "bacterisation"; while, since *Azotobacter chroococcum* is very largely used during this process, and multiplies rapidly in the peat basis, it is probable that the water extract of the material contains a quantity of the products of this organism comparable to the proportions used in the above experiment, since this proportion would amount to only 1·68 per cent. of the weight of the peat.

Effect of Bacillus radicicola.

A further experiment was started in September, 1917, to test the effect of the nitrogen-fixing organism of leguminous nodules—*Bacillus radicicola*—and to compare it with that of *Azotobacter chroococcum*. The rate of growth and multiplication was necessarily slow in this advanced season of the year, and therefore the margin for experimental error became greater, but in spite of these objections fairly uniform results were obtained.

Fifteen dishes were arranged in three series of five dishes each. All contained 150 c.c. of Detmer's solution, and the following additions were made:—Series II, 1 grm. of autoclaved *Azotobacter chroococcum* per 1000 c.c., and Series III, 1 grm. per 1000 c.c. of autoclaved *Bacillus radicicola*, grown in the same manner on maltose-agar plates.

Table IV.

Series.	No.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.	7th week.	8th week.
I.									
Detmer's Solution	1	19	42	80	100	120	160	176	216
	2	21	50	104	140	144	160	200	224
	3	21	43	88	108	136	200	224	240
	4	20	42	92	124	168	184	192	240
	5	21	51	108	132	160	224	208	248
Mean	—	20·4	45·6	94·4	120·8	145·6	185·6	200·0	233·6
II.									
Detmer's Solution + *Azotobacter*	6	24	57	130	196	276	420	556	732
	7	22	57	116	176	232	404	548	672
	8	21	50	118	184	264	416	512	664
	9	20	50	110	172	232	392	500	664
	10	24	59	122	192	276	424	596	740
Mean	—	22·2	54·6	119·2	184·0	256·0	411·2	542·4	694·4
III.									
Detmer's Solution + *Bacillus radicicola*	11	22	50	124	196	296	484	676	852
	12	21	46	104	172	248	412	628	844
	13	22	58	128	196	280	424	616	872
	14	23	57	124	200	288	520	696	928
	15	26	59	128	200	280	464	624	832
Mean	—	22·8	54·0	121·6	192·8	278·4	460·8	648·0	865·6

Ten similar Lemna plants were counted out into each dish, and 300 also thoroughly washed for an estimation of their dry weight. The dishes were

treated precisely as in the preceding experiments with regard to the exclusion of light from bottom and sides and protection from dust. The solutions were changed twice weekly, and the plants counted once weekly, one half of each dish being rejected at the weekly counting when necessary. The numbers obtained are shown in the Table IV.

Estimations of the dry weights of the fractions discarded at the weekly countings were made at the end of the third, fifth, and eighth weeks respectively, with the following results :—

Table V.

Series No.	At beginning.	3rd week.	5th week.	8th week.
I	10·9	10·8	9·9	6·5
II	10·9	12·2	16·0	18·5
III	10·9	12·5	15·9	19·1

These figures indicate that *Bacillus radicicola* is quite as effective as *Azotobacter chroococcum* in promoting the growth of Lemna plants.

Effect of the Ash Constituents.

In all the experiments hitherto described, it has been assumed that it is the *organic* constituents of the additions to the nutrient solutions which have brought about the large increases in growth. There was the possibility, however, that minute quantities of certain *inorganic* substances present in the materials might function as activators, and thus account for the results obtained. In order to test this, fresh quantities of the crude nucleic acid derivatives, and of the *Azotobacter* growth were prepared as above described, in May of 1918, and each was divided into two equal parts. One half of each was carefully evaporated to dryness in a porcelain dish over a water bath, and the residue completely incinerated. When cold the ash was ground to a fine powder with a little water, and carefully transferred to a calibrated stoppered flask, the dish being rinsed with successive small quantities of conductivity water, which were added to the flask. The whole was made up to a known volume, so that an aliquot portion of the well-mixed contents could be taken, representing the ash from a known weight of the original material used.

Twenty-five dishes were then arranged in five series of five dishes each, and all contained 150 c.c. of Detmer's solution. Series I, containing dishes numbered from 1 to 5, constituted the control series, and to the dishes of

the other series the following additions were made: Series II, numbered from 6 to 10, the ash from the crude nucleic acid derivatives from 1 grm. of raw peat per 500 c.c. of solution; Series III, numbered from 11 to 15, the crude nucleic acid derivatives in a similar proportion; Series IV, numbered from 16 to 20, the ash from 1 grm. of *Azotobacter* growth per 1000 c.c.; and Series V, numbered from 21 to 25, 1 grm. of the original *Azotobacter* growth per 1000 c.c. Ten similar plants of *Lemna minor* were counted out into each of the dishes, which were covered on bottom and sides with black paper. Three hundred plants of size comparable with those used in the dishes were counted out at the same time for an estimation of their dry weight. The plants were grown under similar conditions to those recorded in the above experiments, solutions being changed twice weekly while the plants were counted once weekly, and halved or quartered when necessary. The following are the results obtained:—

Table VI.

Series No.	Dish No.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.	7th week.
I. Detmer's Solution	1	37	86	252	464	768	1,344	3,776
	2	34	79	252	480	768	1,408	4,416
	3	34	72	232	496	896	1,536	4,800
	4	34	77	220	480	992	1,536	4,992
	5	33	76	264	464	800	1,408	4,224
Mean	—	34·4	78·0	244·0	476·8	844·8	1,446·4	4,441·6
II. Detmer's Solution + Ash from Nucleic Acid Derivatives	6	32	73	216	472	864	1,536	4,928
	7	46	85	252	528	896	1,408	4,288
	8	46	86	264	448	960	1,536	5,504
	9	40	94	244	528	768	1,472	4,544
	10	35	83	220	464	768	1,408	3,840
Mean	—	39·8	84·1	239·2	488·0	851·2	1,472	4,620·8
III. Detmer's Solution + Nucleic Acid Derivatives	11	33	128	436	1,272	3,136	9,472	34,688
	12	52	132	480	1,128	3,360	8,960	38,144
	13	41	127	432	1,184	3,584	10,176	39,232
	14	42	113	468	1,224	3,424	9,984	39,680
	15	39	117	412	1,208	3,008	9,024	38,912
Mean	—	41·4	123·4	445·6	1,203·2	3,302·4	9,523·2	38,131·2

Table VI—*continued.*

Series No.	Dish No.	1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.	7th week.
IV. Detmer's Solution + Ash from *Azotobacter*	16	37	93	244	480	864	1,344	3,392
	17	36	86	240	536	800	1,280	4,096
	18	37	83	244	472	864	1,472	4,480
	19	37	96	264	480	960	1,472	4,160
	20	39	78	240	488	928	1,472	4,608
Mean	—	37·2	87·2	246·4	491·2	883·2	1,408·0	4,147·2
V. Detmer's Solution + *Azotobacter*	21	40	99	340	872	1,632	4,864	25,216
	22	36	102	340	832	2,048	5,184	26,048
	23	47	102	324	776	1,760	4,928	22,464
	24	48	108	328	800	1,792	4,480	25,024
	25	43	108	324	792	2,048	5,568	28,608
Mean	—	42·8	103·8	331·2	814·4	1,856·0	5,004·8	25,472

The weights of the fractions discarded week by week, as compared with the original weight of the plants, is shown in the following Table:—

Table VII.

Series No.	Weight of 100 plants in milligrams.					
	At beginning.	3rd week.	4th week.	5th week.	6th week.	7th week.
I	14·6	11·2	11·0	10·4	9·6	8·9
II	14·6	11·7	11·3	10·9	10·5	9·4
III	14·6	14·2	15·2	20·6	20·9	20·5
IV	14·6	11·5	10·3	9·7	9·1	8·1
V	14·6	14·3	14·9	18·6	18·8	18·3

The ash of the nucleic acid derivatives and the *Azotobacter* had evidently not the slightest effect on the growth of *Lemna minor*, and the beneficial results following the addition of these materials can only be attributed to their organic constituents.

Conclusion.

The chief interest of the present work centres around two facts: first, that the addition of the derivatives from nucleic acid which has undergone decomposition in decaying vegetable matter—peat—has a marked effect on the growth of Lemna plants in water culture; and second, that the nitrogen-

fixing bacteria have the power of elaborating products, which also greatly increase the growth of the Lemna plants.

All of these organic materials—the crude nucleic acid derivatives and the *Azotobacter* and *Bacillus radicicola* growths—give the Folin-Macullum* reaction, which is stated to indicate the presence of the growth accessory substances—vitamines—which are so important in animal nutrition.

In connection with the effect of the nucleic acid derivatives it is interesting to note that the pure adenine-uracil fraction has not the same effect as the crude extract containing all the products of decomposition. This is in accordance with the work of Schreiner and Skinner,† who found that pure nucleic acid increased the growth of plants in water culture, while some of its derivatives, as hypoxanthine and guanine, though still increasing growth, were not nearly so effective.

Whatever may be the nature of these growth-promoting substances, it is a noteworthy fact that they can be synthesised by the nitrogen-fixing bacteria from a carbohydrate and elementary nitrogen. It remains to be seen whether the products of other bacteria will bring about the same effect, but in working with organisms other than those which fix nitrogen, one great drawback is that a start has to be made by supplying a nitrogenous food material, so that in the bacterial growth there are present not only nitrogenous substances elaborated by the organisms, but also degradation products of this food substance.

It may be that among the products of the nitrogen-fixing bacteria are to be found substances similar in nature to those contained in the crude nucleic acid derivatives from raw peat, and an examination of these products is now in progress to determine whether such substances do occur. In any case, the experiments recorded throw an additional light on the possible *rôle* of the nitrogen-fixing bacteria in the soil, especially in view of the generally accepted fact that the presence of these organisms is always an indication of soil fertility.

The greater part of this work was carried out in 1917, but owing to the author's illness early in 1918 he was unable to complete and publish it until the present time. He wishes to express his sincere thanks to Miss Mockeridge, D.Sc., for her invaluable assistance in the work.

* Folin and Macullum, 'Journ. Biol. Chem.,' vol. 11, p. 265 (1912).

† Schreiner and Skinner, 'U.S. Dept. of Agric., Bureau of Soils,' Bull. No. 87.